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Performance and Egg Quality of Laying Hens Fed Diets Containing Aflatoxin, Fumonisin and Adsorbent

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Adsorbent, aflatoxin, fumonisin, layers, performance.

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ABSTRACT

The effects of aflatoxin and fumonisin and their combination on egg production and quality, as well as the efficacy of a mycotoxin adsorbent in reducing or eliminating these effects in commercial layers. A number of 168 layers with initial age of 37 weeks were submitted to an experimental period of 56 days. A completely randomized experimental design in a 3x2+1 factorial arrangement was applied (3 treatments with mycotoxins: aflatoxin (AF), fumonisin (FU), or aflatoxin + fumonisin (AF+FU); 2 treatments with or without adsorbent; and a control group that was fed no mycotoxins, nor adsorbent), totaling 7 treatments with 6 replicated of 4 birds/cage. The dietary inclusion levels were 1ppm AF, 25ppm FU, and 2 kg adsorbent/ton feed. Birds fed AF presented the lowest percentage of lay ($p=0.0594$). Egg mass was the lowest ($p<0.05$) in the AF+FU treatment (49.49g). The treatment with AF resulted in higher eggshell thickness and strength ($p<0.05$) than the FU treatment and the control group. The inclusion of the adsorbent in the AF contaminated feed reduced eggshell strength, which returned to levels similar to those of the control group. The observed changes indicate that aflatoxin is toxic at a concentration of 1ppm, and that the effects of fumonisin were less evident as a function of the low dose applied. The inclusion of the glucan (2kg/ton) effectively reverted some of the toxic effects of aflatoxin and, at lower extension, those of fumonisin, when these mycotoxins were individually added to commercial layer feeds.

INTRODUCTION

The progress of poultry production is a result of diverse technological developments in nutrition and management techniques. However, significant economic losses may occur due to the presence of natural feed contaminants, such as mycotoxins, which are secondary metabolites produced by fungi that naturally grow in cereals and other grains. Among these, aflatoxins are the most frequently studied and the most widely distributed group of mycotoxins. Aflatoxins are produced by the fungi *Aspergillus flavus*, *A. parasiticus*, and *A. nomius* (Kurtzman *et al.*, 1987), which grow in hot and humid climates, and have mutagenic, carcinogenic, and teratogenic effects (Ellis *et al.*, 1991). Aflatoxins influence the metabolism of poultry, reducing the activity of enzymes that digest starch, proteins, lipids, and nucleic acids, decrease blood protein, total cholesterol, and urea, and increase the activity of serum enzymes that indicate liver damage (Aravind *et al.*, 2003). The main manifestations of chronic aflatoxicosis in layers are reduced egg production and weight, and increase in liver fat levels (Rosmaninho *et al.*, 2001).



Fusarium moniliforme, which can grow both in tropical and temperate climates (Devegowda *et al.*, 1998). Fumonisin is cytotoxic and carcinogenic, and its main mode of action is the inhibition of sphingolipids, which protect the integrity of the cell membrane and play an important role in ion transport through the cell membrane, thereby disturbing cell membrane turnover (Santurio, 2000; Murphy *et al.*, 2006). The main symptoms of fumonisin intoxication in poultry included lower weight gain and increase of kidney and liver weights and hemoglobin concentration (Weibking *et al.*, 1993).

Most studies on feed contamination describe the effects of individual mycotoxins, and do not consider the natural processes where multiple compounds can be produced from a substrate. The interactions among mycotoxins are complex and may cause different effects than those observed when the contamination of individual toxins is evaluated, including additive effects when individual toxins are associated (Andretta *et al.*, 2009). There are few studies establishing the antagonistic, additive, or synergic effects of the exposure to combined mycotoxins (Speijers & Speijers, 2004).

Considering the high contamination of grains worldwide (Council for Agricultural Science and Technology - CAST, 2003) and the problems caused by mycotoxins, one of the alternatives to reduce the damage caused on animal performance is the use of mycotoxin adsorbents. These compounds bind to mycotoxins and prevent their absorption by the animal's gastrointestinal tract, rendering them inert and non-toxic to animals (Krabbe, 1995). One group of mycotoxin adsorbents commonly used in studies carried out with pigs, cattle, and poultry is represented by glucans derived from carbohydrates of the cell wall of some species of yeast (Huwig *et al.*, 2001).

This objective of the present study was to evaluate the adverse effects of aflatoxin and fumonisin and their combination on the egg production and egg quality of commercial layers, and to analyze the efficacy of the mycotoxin adsorbent glucan to reduce and/or to eliminate these effects.

MATERIAL AND METHODS

The experiment was carried out in the environmental chamber of FMVZ, UNESP, Botucatu, Brazil. A number of 168 Hisex® Brown layers were housed at 37 weeks of age in 42 metal cages equipped with

randomized experimental design was applied in a 3x2 + 1 factorial arrangement (three treatments with mycotoxin addition: aflatoxin (AF), fumonisin (FU), and a combination of aflatoxin + fumonisin (AF+FU); with or with no adsorbent; and a control diet with no addition of mycotoxins nor adsorbent), totaling seven treatments with six replicates of four birds each. The experimental unit consisted of a cage with four birds, and the experimental period took 56 days or two 28-d cycles.

Birds were submitted to the same feeding management, with water and feed offered *ad libitum*. Feed was supplied twice daily. Feed intake and egg weight were weekly determined (to calculate egg mass and feed conversion ratio per egg weight and dozen eggs), whereas egg production and percentage of intact eggs were daily determined. Birds were submitted to a photoperiod of 16h of light daily. Average daily temperature was 21.7°C, ranging between 26.4°C and 17.3°C.

The experimental feeds were based on corn and soybean meal, and were formulated according to the nutritional levels recommended by Rostagno *et al.* (2005) for semi-heavy layers, with some modifications following the genetic line manual (Table 1). All feeds were analyzed for the presence of mycotoxins, and the results showed that the control feed was free from mycotoxins, and the contaminated feeds presented aflatoxin and fumonisin concentrations similar to the expected values.

The aflatoxin used in the experiment was produced at the Mycology Laboratory of the Federal University of Santa Maria by fermenting parboiled rice with the fungus *Aspergillus parasiticus*. Fumonisin was produced by the Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, USA, from a corn-based culture of the fungus *Fusarium verticillioides*. The doses used to contaminate feeds were 1ppm aflatoxin and 25ppm fumonisin. The mycotoxin adsorbent used was glucan derived from yeast cell wall at a dose of 2 kg/ton of feed.

A sample of two eggs per replicate was daily collected during the last three days of each 28-d cycle of the experimental period for egg quality assessment. The following parameters were analyzed: average egg weight, eggshell weight, yolk weight, albumen weight and their percentages relative to egg weight, eggshell thickness and strength, albumen height, and Haugh units.

Albumen height was measured using a micrometer,



equation suggested by Stadelman & Cotterill (1995): $HU = 100 \log (H + 7.57 - 1.7 W \times 0.37)$, where H= albumen height (mm), W = egg weight (g), 7.57 = correction factor for albumen height, and 1.7 = correction factor for egg weight.

Table 1 – Ingredient and estimated nutrition composition of the experimental diets.	
Ingredients	Inclusion level (%)
Corn	57.26
Soybean meal (45)	29.15
Soybean oil	2.05
Salt	0.35
Fine calcitic limestone	6.30
Coarse calcitic limestone	2.70
Dicalcium phosphate	1.55
DL-methionine, 99%	0.14
Vitamin supplement ¹	0.10
Mineral supplement ²	0.10
Sodium bicarbonate	0.10
Rice straw or adsorbent	0.20
TOTAL	100.00
Calculated values	
Metabolizable energy, kcal/kg	2.780
Crude protein, %	18.02
Calcium, %	3.92
Available phosphorus, %	0.39
Methionine, %	0.42
Met+Cys, %	0.71
Lysine, %	0.94
Sodium, %	0.18
Chlorine, %	0.25
Linoleic acid, %	2.35
Tryptophan, %	0.22
Threonine, %	0.70
1 - Vitamin supplement (Postura C, Multimix). Composition per kg product: vitamin A 7,000,000IU, vitamin D 2,000,000IU, vitamin E 5,000mg, vitamin K3 1,800mg, vitamin B2 3,000mg, vitamin B12 8,000mcg, niacin 20,000mg, pantothenic acid 5,000mg, antioxidant 15,000mg, and carrier QSP 1,000g. 2 - Mineral supplement (Multimineral aves, Multimix). Composition per kg product: copper 8,000mg, iron 50,000mg, manganese 70,000mg, zinc 50,000mg, iodine 1,200mg, selenium 200mg, and carrier QSP 1,000g.	

Specific egg gravity was determined according to the method described by Stadelman & Cotterill (1995), using saline solutions with densities between 1.065 and 1.100. Eggshells remained in the oven for three days at 60°C to calculate eggshell percentage. Yolk

Eggshell thickness was measured using a micrometer. The strength eggshell to break was evaluated using a specific cell coupled to the apparatus Texture Analyser TA XT plus, and the probe Cyl Stainless 2mm recorded the strength required to break the eggshell in kgf.

Data were submitted to analysis of variance by the General Lineal Model procedure (GLM) with the aid of Statistical Analysis System software package (SAS, 2002). When the F test yielded significant results ($p < 0.05$), treatment means were compared by the tests of Tukey and Dunnett. The test of Tukey was applied to compare treatment means and the test of Dunnett was used to compare the mean of the control treatment with those of the other treatments.

RESULTS AND DISCUSSION

During the entire experimental period, no visible changes in bird general health were observed, and only one bird, belonging to treatment AF, died. Table 2 shows the mean values of feed intake, percentage of lay, feed conversion ratio, egg weight, egg mass, and intact egg percentage of the different treatment groups. No interactions ($p > 0.05$) between mycotoxins and adsorbent were observed for none of the evaluated performance parameters (Table 2) and nor treatment effects ($p > 0.05$) on feed intake, feed conversion ratio, or mean egg weight.

The concentrations of mycotoxins used in the present study did not change the acceptability of the contaminated feeds as there were no differences in feed intake among treatments. There are few reports in literature on the effects of mycotoxins on feed intake, making it difficult to compare results. Oliveira *et al.* (2001) did not observe any feed intake differences in layers fed diets containing 300 and 500ug AF/kg. On the other hand, Hamilton & Garlich (1971) found reduced feed intake in layers when diets were contaminated with 1.25 to 20.0mg/kg AF. Abreu *et al.* (2004) verified feed intake reduction in quails fed levels higher than 200µg AF/kg in the feed, and Ledoux *et al.* (1992) also recorded reduced feed intake in broilers for FB₁ concentrations between 100 and 400 mg/kg.

Percentage of lay of birds fed the diet containing AF+FU and adsorbent was lower (73.51%) as compared to that of the control group (94.79%, Table 2). Birds fed AF+FU presented lower ($p = 0.0594$) percentage of lay (75.45%) than those in the FU group (88.77%). The reduction of egg production observed in the present study was probably a result of the hepatotoxic



effects of fumonisin on protein and lipid metabolism, impairing egg synthesis. Reduced egg production was also reported by Oliveira *et al.* (2002) for dietary levels of 100, 300, and 500 µg AF/kg feed, as well as by Hamilton & Garlich (1971), Iqbal *et al.* (1983), and Sudhakar (1990) for AFB1 concentrations higher than 600 µg/kg. Kubena *et al.* (1999), observed reduced egg production when feeding layers with a feed containing 200mg FU/kg.

Feed conversion ratio per kg (FCR/kg) and per dozen eggs (FCR/dz) were not influenced by the treatments (Table 2). These results are consistent with those obtained by Iqbal *et al.* (1983), who fed aflatoxin at the same concentration as that used in the present study. However, those authors observed worse feed conversion ratio when feeding layers with 2mg/kg aflatoxin, as well as by Oliveira *et al.* (2001), using 500 µg/kg.

Mean egg weight (Table 2) was not statistically different among treatments; however, the lowest weight was obtained with the AF+FU treatment (65.03g) as compared to those containing a single

mycotoxin (68.06g and 68.48g for AF and FU, respectively). Despite not statistically significant ($p>0.05$), this result indicates a possible additive and negative effect of mycotoxins on egg weight. Oliveira *et al.* (2001) did not find any differences in the average egg weight of layers fed 500 µg/kg AF. Washburn *et al.* (1985) observed a reduction in the egg weight of layers fed for three weeks with a feed contaminated with 5mg/kg AF, that is, five times the aflatoxin concentration used in the present study.

Kubena *et al.* (1999) evaluated the effects of the chronic administration of FB1 at 100 and 200mg/kg feed and found higher egg weight in layers fed 100mg FU/kg after the ninth 28-d period of intoxication. Butkeraitis (2003) observed reduced egg weight in quails fed 50 and 250mg FU/kg feed as compared to the control treatment and concluded that the effect of mycotoxins on egg weight is dependent of dose.

The treatment AF+FU with adsorbent resulted in lower egg mass as compared to the control group (47.95g and 64.06g egg/bird/day, respectively). Egg mass was reduced in the treatments containing

Table 2 – Performance parameters of commercial layers submitted to different treatments.

Treatments ¹	CONT	ADS	Mycotoxins			Mean	p-value				CV (%)
			AF	FU	AF+FU		ADS	MYCO	INT	CONT	
Feed intake (g/bird/day)	113.57	Without	119.40	116.58	115.22	117.08	0.2069	0.5111	0.7427	0.6247	7.44
		With	113.57	115.98	110.05	113.20					
		Mean	116.49	116.28	112.65						
% of lay	94.79	Without	76.72	86.76	77.38	80.29	0.5828	0.0594	0.5793	0.0430	14.77
		With	83.93	90.77	73.51*	82.74					
		Mean	80.33 ab	88.77 a	75.45 b						
Feed conversion ratio/kg egg	2.17	Without	1.93	2.21	2.13	2.09	0.4874	0.2239	0.9458	0.6983	22.10
		With	1.97	2.35	2.29	2.20					
		Mean	1.95	2.28	2.21						
Feed conversion ratio/dz eggs	1.77	Without	1.60	1.78	1.68	1.69	0.4915	0.2624	0.7451	0.6699	22.54
		With	1.56	1.90	1.89	1.78					
		Mean	1.58	1.83	1.79						
Mean egg weight (g)	67.59	Without	67.90	69.17	65.33	67.47	0.6681	0.0665	0.8582	0.4376	5.84
		With	68.23	67.79	64.74	66.92					
		Mean	68.06	68.48	65.03						
Egg mass (g egg/bird/ day)	64.06	Without	51.75	60.88	51.02	54.55	0.7336	0.0198	0.5465	0.0263	16.14
		With	57.40	61.62	47.95 *	55.66					
		Mean	54.58 ab	61.25 a	49.49 b						
% intact eggs	94.72	Without	97.02	97.32	96.87	97.07 A	0.0438	0.7563	0.6047	0.4278	4.02
		With	93.97	93.23	95.91	94.37 B					
		Mean	95.50	95.27	96.39						

1 - Treatments: CONT: control, AF: aflatoxin, FU: fumonisin, AF+FU: aflatoxin plus fumonisin. ADS: adsorbent. MYCO: mycotoxin. INT: interaction. *



mycotoxins (Table 2), and the lowest mean ($p<0.05$) was obtained when mycotoxins were combined (49.49g egg/bird/day). These results demonstrate the additive effect of mycotoxins and that the adsorbent was not effective in preventing the intestinal absorption of these toxins.

In the treatments containing mycotoxins, but no adsorbent, intact egg percentage was (97.07%) as compared to the treatments that contained the adsorbent (94.37%). The eggs of the treatments containing mycotoxins presented higher eggshell strength (Table 3), which was not the case when the adsorbent was present, which treatments presented lower percentages of intact eggs.

External and internal egg parameter results are presented in Table 3. There was effect ($p<0.05$) of the interaction between mycotoxins and adsorbent inclusion on egg specific gravity. Layers fed diets containing FU and no adsorbent presented lower egg

specific gravity ($p<0.05$) as compared to those in the AF treatment (1.087 and 1.091, respectively). Oliveira *et al.* (2001) did not find any differences in egg specific gravity values of birds fed aflatoxins at 100, 300, or 500 μ g/kg, which were maintained around 1.090. In the present study, the treatment containing AF plus adsorbent resulted in the lowest ($p<0.05$) egg specific gravity. Apparent egg density or egg specific gravity is frequently used in the evaluation of egg quality as it is obtained using a fast, convenient, and low cost method. This characteristic is directly related to eggshell percentage (Hamilton, 1982), because the higher eggshell thickness, the higher egg relative density (Butkeraitis, 2003).

Eggshell thickness (Table 3) was higher in the eggs of birds fed AF and no adsorbent (0.43mm) relative to the control group (0.41mm). The eggs of layers fed AF presented higher ($p<0.05$) eggshell thickness than those submitted to the FU and AF+FU treatments.

Table 3 – Egg quality parameters in commercial layers submitted to different treatments.

Treatments ¹	CONT	ADS	Mycotoxin			Média	p-value				CV (%)
			AF	FU	AF+FU		ADS	MYCO	INT	CONT	
Specific gravity (g/L)	1.098	Without	1.091 aA	1.087 b	1.091 a	1.089	0.8334	0.0613	0.0255	0.5676	0.87
		With	1.089 B	1.091	1.090	1.090					
		Mean	1.090	1.088	1.091						
Eggshell thickness (mm)	0.41	Without	0.43*	0.41	0.42	0.42	0.6398	0.0448	0.2346	0.0558	3.27
		With	0.42	0.42	0.42	0.42					
		Mean	0.43 a	0.41 b	0.41 b						
Eggshell %	9.13	Without	9.41 ab	8.96 bB	9.74*a	9.37	0.9008	0.0425	0.0129	0.0161	3.80
		With	9.21	9.46 A	9.40	9.36					
		Mean	9.31	9.21	9.57						
Eggshell strength (kgf)	3.03	Without	3.41 *aA	2.80 b	3.22 a	3.14	0.2696	<0.0001	0.0220	0.0005	6.56
		With	3.15 B	2.98	3.09	3.07					
		Mean	3.28	2.89	3.16						
Yolk %	23.35	Without	22.57	23.19	21.84	22.54	0.8663	0.0014	0.3973	0.0590	4.40
		With	22.04	23.76	21.97	22.59					
		Mean	22.31 a	23.48 a	21.91 b						
Albumen %	67.51	Without	68.01	67.84	68.41	68.09	0.9079	0.0251	0.1467	0.0741	1.70
		With	68.74	66.78	66.62	68.05					
		Mean	68.38 ab	67.31 b	68.51 a						
Haugh units	80.00	Without	82.45	81.84	86.13*	83.47	0.8812	0.0129	0.7013	0.0373	4.52
		With	83.61	80.38	85.86*	83.28					
		Mean	83.03 ab	81.11 b	85.99 a						
Yolk color	5.36	Without	5.74*	5.46	5.96*	5.72	0.0685	0.0185	0.1058	0.0015	3.99
		With	5.54	5.54	5.62	5.57					
		Mean	5.64 ab	5.50 b	5.79 a						

1 - Treatments: CONT: control, AF: aflatoxin, FU: fumonisin, AF+FU: aflatoxin plus fumonisin. ADS: adsorbent. MYCO: mycotoxin. INT: interaction. * -



Galkate & Rokde, (2010) observed higher eggshell thickness in layers fed diets containing 0.5, 1.0, and 2.0mg aflatoxin/kg. In quails intoxicated with 50mg/kg FB1, Butkeraitis (2003) verified higher eggshell thickness.

The interaction between mycotoxins and adsorbent inclusion influenced ($p<0.05$) eggshell percentage. The addition of adsorbent to the diet increased eggshell percentage in layers fed FU (9.46 and 8.96 % for the treatments without and with adsorbent, respectively). Eggshell percentage was higher in birds of the AF+FU treatments (9.74%) as compared to those in the FU treatment (8.96%) when no adsorbent was added to the diet. Higher eggshell percentage ($p<0.05$) was also observed in the eggs of the layers submitted to the AF+FU treatment (9.74%) as compared to the control group (9.13%). This result is consistent with the findings of Devegowda & Ravikiran (2008), who verified a correlation between AFB1 and reduced egg quality. Oliveira *et al.* (2001) reported that eggshell percentage was not affected by AFB1 treatments (100, 300, or 500 mg/kg), whereas Zaghini *et al.* (2005) observed reduced eggshell weight when birds were fed 2.5 mg/kg AFB1.

The interaction between mycotoxins and adsorbent inclusion ($p<0.05$) affected eggshell strength. The AF treatment resulted in higher eggshell strength (3.41kgf), as compared to the control group (3.03kgf) and to the FU treatment (2.80kgf) with no inclusion of adsorbent. Washburn *et al.* (1985), found higher eggshell strength in layers intoxicated with aflatoxins. The inclusion of adsorbent in the AF treatment reduced eggshell strength, showing that the added glucan effectively reduced the intestinal absorption of aflatoxin, preventing its effect of increasing eggshell strength.

The interaction between mycotoxins and adsorbent inclusion did not significantly ($p>0.05$) influenced yolk and albumen percentages, Haugh units, or yolk color (Table 3).

Yolk percentage was lower ($p<0.05$) in the AF+FU treatment, and no differences were observed between the treatments containing individual toxins, which suggests an additive effect on this parameter. Literature shows that aflatoxin, in addition of reducing egg production, also causes yolk size reduction due to its interference on the liver metabolism of proteins and lipids, which are the main yolk components (Huff *et al.*, 1975; Vieira, 1995). The intoxication of birds with aflatoxins causes fat deposition in the liver, impairing lipid mobilization to the ovarian follicles, and

The combination of mycotoxins caused an increase ($p<0.05$) in albumen percentage as compared to the FU treatment (Table 3), as a result of the reduction of yolk percentage caused by mycotoxins.

Haugh units (HU) were higher ($p<0.05$) in the AF+FU treatment (85.99) as compared to the treatment containing only FU (81.11) and to the control group (80.00), evidencing the additive effect when mycotoxins are associated (Table 3). Oliveira *et al.* (2001) observed in layers contaminated with 300 and 500 μg aflatoxin/kg feed HU values lower relative to non-contaminated control, despite the lack of statistical differences ($p>0.05$). Galkate & Rokde (2010) did not find different HU values in layers contaminated with 0.5, 1.0, or 2.0mg aflatoxin/kg feeds. In quails fed aflatoxin at 25, 50 and 100 $\mu\text{g}/\text{kg}$ feed (Oliveira, 2001) and 1000 and 2000 $\mu\text{g}/\text{kg}$ feed (Abreu *et al.*, 2008), no HU differences were detected HU.

Yolk color was higher ($p<0.05$) in the egg of layers submitted to AF treatments as compared to the control group (Table 3). Huff *et al.* (1975) found higher yolk color in birds intoxicated with 10 μg of AFB1/kg. Yolk color variation is related to the interference of aflatoxin in lipid metabolism (Tung *et al.*, 1972; Vieira, 1995) and in carotenoid absorption or their deposition in the yolk (Genedy *et al.*, 1999). In the present study, it was not demonstrated that mycotoxins changed the intestinal absorption of carotenoids, as the yolk color of the eggs of the intoxicated presented higher value relative to the control group. The reduction of yolk size of layers fed mycotoxins possibly contributed to concentrate carotenoids in the yolk, resulting in higher color intensity.

The lower yolk size of the eggs of birds fed aflatoxin in the present study was compensated for the increase in the size of albumen and eggshell percentage, and therefore, there were no differences in mean egg weight. Washburn *et al.* (1985), using 5mg/kg AFB1, observed reduction in egg size and increase in eggshell percentage, which was explained by the fact the aflatoxins are metabolized in the liver, where the synthesis and transport of the precursors required to yolk production occur.

CONCLUSIONS

In the present study, the changes in the production performance of layers and in egg quality indicate that aflatoxin is toxic at a concentration of 1mg/kg. Despite increasing eggshell strength, aflatoxin reduces egg



value. The effects of fumonisin were less evident due to the low dosage used in the present study (25mg/kg). The mycotoxin adsorbent glucan at a concentration of 2kg/ton effectively reverted some of the toxic effect of aflatoxin and, in lower extension, of fumonisin, when these mycotoxins were individually added to the feed of commercial layers.

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